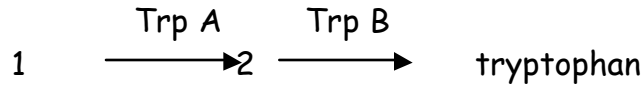


7.012 Section Problem: Complementation

1) Consider the following hypothetical pathway for the synthesis of the amino acid tryptophan.

- Compound 1 is converted to compound 2 by the enzyme encoded by the *trpA* gene.
- Compound 2 is converted to tryptophan by the enzyme encoded by the *trpB* gene.



You also have some mutants, t1 through t5:

- t1, t2, and t4 are in the *trpA* gene
- t3 and t5 are in the *trpB* gene

a) What is the phenotype of each of the mutants?

b) Suppose that you construct a diploid strain that contains mutant t1 on one chromosome and t2 on the other chromosome. Assuming that the phenotype of each of these mutants is recessive to wild type (one good copy of a gene is sufficient for wild-type phenotype), what will be the phenotype of this diploid?

c) Suppose that you construct a diploid strain that contains mutant t1 on one chromosome and t3 on the other chromosome. Assuming that the phenotype of each of these mutants is recessive to wild type (one good copy of a gene is sufficient for wild-type phenotype), what will be the phenotype of this diploid?

d) Based on your answers to parts (b) and (c) complete the following chart.

- Put a "+" in the box if the resulting diploid can synthesize tryptophan (wild-type phenotype)
- Put a "-" in the box if the resulting diploid cannot synthesize tryptophan (mutant phenotype)

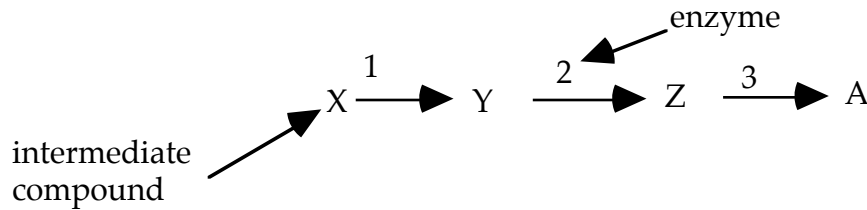
	t1	t2	t3	t4	t5	wt
t1						
t2						
t3						
t4						
t5						
wt						

e) Suppose you had a mutant t6 that was in gene *trpA* and had a dominant phenotype; that is, a diploid with this genotype : $\frac{t6}{+}$ is unable to synthesize tryptophan. Complete the following chart.

	t1	t2	t3	t4	t5	t6	wt
t6							

7.012 Epistasis and Complementation

1) Given the following pathway for the synthesis of the essential compound A:



Mutants with defects in genes 1, 2, or 3 (m1, m2, m3) will require A to grow on minimal medium.

a) What intermediate will build up in the following mutants:

m1:

m2:

m3:

b) What intermediate(s) will the following mutants grow on?

m1:

m2:

m3:

c) What intermediate will build up in the following double mutants?

m1,m2:

m2,m3:

m1,m3:

d) What intermediate(s) will the following double mutants grow on?

m1,m2:

m2,m3:

m1,m3:

2) You are studying the biosynthesis of the amino acid arginine. You have mutants each with a mutation in one of four genes (m1, m2, m3, and m4) and three potential intermediates (A, B, C). You plate your mutants on plates with the following media and see if they grow (+) or not (-).

Strain	Medium				
	min	min + A	min + B	min + C	min + arg
wild-type	+	+	+	+	+
m1	-	-	+	-	+
m2	-	-	-	-	+
m3	-	+	+	-	+
m4	-	+	+	+	+

a) What is the order of enzymes and intermediates in the pathway?

b) With what supplement(s) would the double mutant m1,m4 grow?

3) You are studying the pathway of pigment synthesis in the fictitious bacterium, *Bacterium colorificus*. Wild-type bacteria are red. You have three mutant strains, with altered colors.

<u>Genotype</u>	<u>Color Phenotype</u>
m1 ⁺ m2 ⁺ m3 ⁺	red (wild-type)
m1 ⁻ m2 ⁺ m3 ⁺	orange
m1 ⁺ m2 ⁻ m3 ⁺	yellow
m1 ⁺ m2 ⁺ m3 ⁻	colorless
m1 ⁻ m2 ⁻ m3 ⁺	orange
m1 ⁺ m2 ⁻ m3 ⁻	colorless
m1 ⁻ m2 ⁺ m3 ⁻	colorless

Based on this information, determine the order of the enzymes and intermediates in this pathway.

4) You are studying a bacteriophage and trying to determine how many genes are required for the phage to infect and lyse the host bacterium. You have eight mutants, all of which are unable to lyse a particular host bacterium. (Note: there must be another host that can be lysed by these mutants, otherwise, you couldn't grow them!) You perform pairwise infections with each of your mutant strains and get the following result: (+) = pair of phages lysed bacterium, (-) = pair of phages failed to lyse bacterium.

	m1	m2	m3	m4	m5	m6	m7	m8	wt
m1	-	+	+	-	-	+	+	+	+
m2		-	+	+	+	+	+	+	+
m3			-	+	+	-	-	-	+
m4				-	-	+	+	+	+
m5					-	+	+	+	+
m6						-	-	-	+
m7							-	-	+
m8								-	+
wt									+

a) What are the complementation groups and how many genes have you identified?

b) Can you be sure that you have identified all the genes involved?